SFES, FIR 2203
2500 Shreveport Highway
Pineville, LA 71360

Don —
Return to Donna for files.
Pete

4500

July 1, 1985

Forest Insect Research

RAK

Bob Joslin, Forest Supervisor
through
Pete Lorio, Project Leader Jm

In connection with enclosed Study Plan, FS-SO-2203-3.01, I need to determine which species of sawyer beetles are capable of vectoring pine wood nematodes and when the nematode is most abundant in the beetle population. It will be necessary to trap beetles in window traps baited with an attractant. The attractant used will not be the frontalin and alpha-pinene attractant used to lure the southern pine beetle, but will be a combination of ipsdienol and ipsenol known to attract only *Ips* engraver beetles and at least one species of sawyer beetles (Billings and Cameron, 1984).

Permission is requested to place several baited window traps in the Livingston area of the Catahoula Ranger District. These traps can be placed in predominantly hardwood areas provided they are in the proximity of dead pines (logging debris, old southern pine beetle spots, etc.). I would like to sample throughout the summer and fall months until sawyer emergence ceases.

D. N. KINN
Research Entomologist
Forest Insect Research

Enclosure

cc: AD-W

Approved by Barrett + Bobby Cox 7/24/85

STUDY PLAN

INCIDENCE OF PINE WOOD NEMATODES IN BARK BEETLE ATTACKED TREES[1]

D. N. Kinn

Research Entomologist

FS-SO-2203-3.01

[1] Study listed on page 20 of Problem Analysis FS-SO-2203-3.0

4500
FS-SO-2203-3.01
(Problem 3)

STUDY PLAN

INCIDENCE OF PINE WOOD NEMATODES IN BARK BEETLE ATTACKED TREES

Prepared by:

D. N. Kinn
Research Entomologist

4-19-'83
Date

Approved:

Peter L. Lorio, Jr.
Project Leader
Forest Insect Research

4-19-83
Date

S. J. Barras
Assistant Director-West
Southern Forest Experiment Station

4/25/83
Date

Addendum to FS-SO-2203-3.01

Adult sawyer beetles (longhorn beetles) are attracted to dead and dying pines on which they mate and eventually deposit eggs in crater-like cavities chewed through the outer bark. Pine wood nematodes can be introduced into dead trees through these oviposition wounds just as they can be introduced into healthy trees through feeding wounds made by the beetle. The presence of these nematodes in southern pine products, especially wood chips, has recently become a problem to exporting companies.

To determine which species of sawyer beetles vector this pathogenic worm and to determine when during the year the nematode is vectored in greatest numbers, sawyer beetles flying to host trees will be trapped and examined for nematodes. Window traps baited with Ips attractant will be placed near areas where sawyer beetle populations are expected to exist. At least one species of sawyer beetle is known to be attracted to Ips attractant (Billings and Cameron, 1984). Since, as far as is known, trees used for maturation feeding are selected randomly by the beetle, and no feeding attractant is known, it is not possible to trap recently emerged beetles.

4500
FS-SO-2203- 3.01
(Problem 3)

April 1983
Study Plan Summary
D. N. Kinn - Alexandria, La.

INCIDENCE OF PINE WOOD NEMATODES IN BARK BEETLE ATTACKED TREES

Trees attacked by bark and engraver beetles will be sampled for the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, and the degree of coincidence of scolytids and nematodes determined to ascertain whether or not pine wilt disease might be a potential stress factor predisposing trees to attack by scolytids.

INTRODUCTION

The pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (= *B. lignicolus* Mamiya and Kiyohara) is either directly or indirectly the causative agent of pine wilt disease. Disease symptoms may be the result of phytotoxins produced by the nematode (Dropkin, et al. 1981, Bolla, Shaheen and Winter 1982), or a toxin produced by a bacterium, *Pseudomonas* sp., carried into the tree by the worm (Oku, et al. 1980). *B. xylophilus* utilizes fungus hyphae grown on wood tissue as its food (Kobayashi, Sasaki and Mamiya 1975). The fungi include species belonging to the genera *Ceratocystis, Pestalotis, Macrophoma, and Fusarium.*

In Japan, where pine wilt disease has been devastating forests for the past 30 years (Nickle 1981, Wingfield, et al. 1982a), the nematode is vectored by the Japanese sawyer beetle, *Monochamus alternatus* Hope. (Ishibashi and Kondo 1977). The life cycle of *B. xylophilus* is closely synchronized with that of its phoretic host and a truely remarkable mutualism exists among the sawyer beetle, PWN, and the various bacteria and fungi associated with them. The J_4 dauerlarva is transported to new hosts by entering the tracheae of callow adult beetles by way of the spiracles (Mamiya 1972, Togashi and Sekizuka 1982). The dauerlarvae of *B. xylophilus* and microorganisms are introduced into healthy trees through wounds made during maturation feeding of the beetle, and the resulting pine wilt disease stresses or kills the tree making it suitable for cerambycid oviposition and larval development (Dropkin, et al. 1981).

The pine wood nematode (= timber nematode) was originally found in Louisiana in 1931 and it is now believed that it was exported to Japan (Wingfield, et al 1982a). Damage caused by the pine wood nematode in the United states during the past 50 years was probably attributed to insects

and fungi (Nickle 1980). Native North American species such as long leaf pine (*Pinus palustris* Mill.), loblolly pine (*P. taeda* L.) and shortleaf pine (*P. echinata* Mill.) are partially resistant whereas many exotic species are susceptable to pine wilt disease (Futai and Furuno 1979, Mamiya 1976). The pine wood nematode is known to be present in 32 states and has been collected from 20 species of pine as well as from Atlantic cedar (*Cedrus atlantica* Manetti), deodar cedar (*C. deodara* (Roxb. Loud.)), European larch (*Larix decidua* Mill.), tamarack ((*L. laricina* Du Roi) K. Koch), and white spruce (*Picea glauca* Moench. Voss) (Dropkin, et al. 1981, Wingfield, et al. 1982a).

Pine wilt disease, in general, is endemic in the United States and not, at present, epidemic (Dropkin, et al. 1981) except in limited areas on introduced species (Adams and Morehart 1982). Damage is usually restricted to individual trees or plantations (Nickle 1982). However, the presence of pine wilt disease in the United States, especially in the southern states, has caused concern regarding the potential that this disease could inflict over the extensive pine stands now existing in the south (Anon. 1982, Dropkin, et al. 1981). Should this disease become epidemic in the United States, there is no known effective control. In Japan, pesticides are being applied to control the cerambycid vector, with limited success.

Scotch pine Xmas tree plantations ?

OBJECTIVES

The recent discovery of the pine wood nematode in the United States raises a number of questions regarding the interrelationships among nematodes, pine wilt disease, cerambycids and scolytids. Wingfield, et al. (1982b) only found *B. xylophilus* in trees colonized by suppossed secondary insects such as bark beetles and borers. They speculated that the pine wood nematode may only become established in stressed trees. However, scolytids and cerambycids

usually do not attack healthy pines and oviposit only in weakened or dead trees (Morimoto and Iwasaki 1972). Scolytids are not known to vector the pine wood nematode (Mamiya 1972, Kakuliya 1966). Therefore, the possibility exists that bark and engraver beetles may exploit a resource provided by cerambycids and the pine wood nematode. This study will determine the incidence of pine wilt disease in scolytid infestations.

METHODS AND MATERIALS

Trees determined to be the original tree attacked in a bark beetle infestation will be sampled for pine wood nematodes. Xylem samples will be taken from the bole by removing the outer bark, swabbing the exposed xylem with 70% ethanol, and drilling one inch deep into the xylem using a 3/4" wood bit. Samples will be taken from several portions of the bole and wood cuttings caught in Ziploc® sandwich bags, and transported to the laboratory in an ice chest. In the laboratory, some of the woodcuttings will be wrapped in Kimwipes® and soaked for 24 hours in distilled water. The water will then be examined for nematodes and any found transferred to a drop of 3% formalin on a glass slide and identified under a compound microscope. Additional xylem samples will be transferred to petri dishes containing the fungus *Monilinia fructicola* (Winter) Honey cultured on potato dextrose agar. After two weeks of incubation at 25°C. the petri dishes will be examined for nematodes and those found transferred to temporary microscope mounts and identified.

Data taken from stands in which trees have been sampled for PWN will include: forest type, stand age, site index, and hazard class. Independence of pine species and the incidence of PWN will be tested using Chi-square tests, and forest type, stand age, etc. of diseased and non-diseased trees will be compared using t-tests.

APPLICATION OF RESEARCH RESULTS

Results of this study will be presented in a final report consisting of a manuscript or published paper. This study should provide data on which to base a decision for conducting future research on the susceptibility of southern pines to pine wilt disease.

SAFETY AND HEALTH CONSIDERATIONS

This study presents minimal risks to the safety and health of the investigators. No extraordinary safety measures need to be adressed other than safe work practices standard to the laboratory environment.

ENVIRONMENTAL CONSIDERATIONS

The research activities described here are limited in scope and will have no impact in terms of changes in the physical, biological, economic, or social components of the environment thereby qualifying as a categorical exclusion (FSM-1952).

PERSONNEL ASSIGNMENT

Donald N. Kinn	Research Entomologist
William J. Oakes	Biological Technician

This study will be initiated in 1983 and is expected to cover a period of three years, terminating in 1986.

ESTIMATED EXPENSES

Labor (wages)	$15,000
Travel	$4,000
Equipment	
Nonexpendable	
Microscope	$3,000

Expendable

Glassware	$2,000
Culture media	$1,000
Hand tools	$500
Misc. expenses	$3,000

COOPERATIVE RESEARCH

Field data (site, stand and tree characteristics) required for another study (FS-SO-2203-6.15) will be collected by Erich G. Vallery and temporary employees under the supervision of Peter L. Lorio, Jr. and Robert A. Sommers. Xylem samples will be taken from trees in selected plots when feasible.

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Addendum to FS-SO-2203-3.01

Adult sawyer beetles (longhorn beetles) are attracted to dead and dying pines on which they mate and eventually deposit eggs in crater-like cavities chewed through the outer bark. Pine wood nematodes can be introduced into dead trees through these oviposition wounds just as they can be introduced into healthy trees through feeding wounds made by the beetle. The presence of these nematodes in southern pine products, especially wood chips, has recently become a problem to exporting companies.

To determine which species of sawyer beetles vector this pathogenic worm and to determine when during the year the nematode is vectored in greatest numbers, sawyer beetles flying to host trees will be trapped and examined for nematodes. Window traps baited with Ips attractant will be placed near areas where sawyer beetle populations are expected to exist. At least one species of sawyer beetle is known to be attracted to Ips attractant (Billings and Cameron, 1984). Since, as far as is known, trees used for maturation feeding are selected randomly by the beetle, and no feeding attractant is known, it is not possible to trap recently emerged beetles.

Kairomonal Responses of Coleoptera, *Monochamus titillator* (Cerambycidae), *Thanasimus dubius* (Cleridae), and *Temnochila virescens* (Trogositidae), to Behavioral Chemicals of Southern Pine Bark Beetles (Coleoptera: Scolytidae)

RONALD F. BILLINGS AND R. SCOTT CAMERON

Forest Pest Control Section, Texas Forest Service,
Lufkin, Texas 75901

Environ. Entomol. 13: 1542–1548 (1984)

ABSTRACT The kairomonal responses of the sawyer beetle, *Monochamus titillator* (F.), and two predators, *Thanasimus dubius* (F.) and *Temnochila virescens* (F.), to southern pine bark beetle attractant and inhibitor mixtures were monitored in a series of replicated field bioassays. Tests were conducted in the presence and absence of measurable populations of the southern pine beetle, *Dendroctonus frontalis* Zimmermann. At three different test sites, *M. titillator* responded to traps baited with pheromone mixtures attractive to southern *Ips* spp. (*I. avulsus* [Eichhoff], *I. grandicollis* [Eichhoff], *I. calligraphus* [Germar]), particularly those also containing *D. frontalis* inhibitor. This sawyer exhibited a reduced numerical response to a combination of *D. frontalis* attractant and *D. frontalis* inhibitor, but no significant response to traps baited solely with *D. frontalis* attractant. This is the first report of a sawyer beetle responding to pheromones of coinhabiting pine bark beetles. The predator *T. dubius* was captured in greatest numbers in traps baited with *D. frontalis* attractant, and in significantly lesser numbers in traps containing *Ips* attractant. In contrast to *T. dubius*, the predator *T. virescens* responded almost exclusively to *Ips* attractant and showed little response to *D. frontalis* attractant. These kairomonal responses explain previously observed temporal and spatial arrival patterns of these insect associates to bark-beetle-infested southern pines.

PINES COLONIZED BY the southern pine beetle (SPB), *Dendroctonus frontalis* Zimmermann, are coinhabited by a variety of other insects which may include three species of southern pine engraver beetles (*Ips avulsus* [Eichhoff], *I. grandicollis* [Eichhoff], and *I. calligraphus* [Germar]), as well as parasites, predators, and other associates. Temporal and spatial patterns of host tree colonization by sympatric bark beetle species are mediated by a system of insect- and host-produced behavioral chemicals. Both male and female *D. frontalis* aggregate on host trees in response to the female-produced pheromone frontalin, in combination with host-produced volatile chemicals, particularly alpha-pinene. Males of *D. frontalis* produce verbenone and *endo*-brevicomin which act as inhibitors to reduce the attraction of additional beetles to the host tree (Renwick and Vité 1970, Payne 1980).

Southern *Ips* species, which may colonize pines initially attacked by *D. frontalis*, are known to respond to different behavioral chemicals. *Ips avulsus* aggregates in response to ipsdienol and (S)-(−)-ipsenol, while *I. calligraphus* uses (S)-*cis*-verbenol and ipsdienol as an aggregation pheromone. In turn, *I. grandicollis* produces and responds to (S)-(−)-ipsenol as its only pheromonal compound (Vité et al. 1978). Synthetic mixtures of frontalin and alpha-pinene, and of *endo*-brevicomin and verbenone, have been used in experimental trials to manipulate SPB populations (Vité and Francke 1976, Richerson and Payne 1979, Richerson et al. 1980) and offer promise for use in future pest management tactics. Also, traps baited with a combination of synthetic ipsdienol, ipsenol, and *cis*-verbenol have been used to collect all three species of southern *Ips* (Rose et al. 1981).

Behavioral chemicals produced by southern pine bark beetles serve as kairomones for many species of predaceous and parasitic insects (Dixon and Payne 1980). For example, the predator *Thanasimus dubius* (F.) simultaneously locates adult bark beetles for food and suitable habitat for oviposition (e.g., beetle-killed pines) by responding to frontalin produced by attacking *D. frontalis* females (Vité and Williamson 1970, Dixon and Payne 1980, Payne et al. 1984). The kairomonal responses of other associated insects, however, need to be elucidated before synthetic pheromones can be incorporated into effective pest management tactics.

The purpose of this paper is to report the responses of the sawyer, *Monochamus titillator* (F.), and the two predators, *T. dubius* and *Temnochila virescens* (F.), to those formulations of synthetic attractants and inhibitors known to have potential for bark beetle manipulation in southern pine for-

ests. With the exception of the attraction of *T. dubius* to the *D. frontalis* aggregating pheromone frontalin (Vité and Williamson 1970, Payne et al. 1984), little is known about the kairomonal responses, if any, of these common associated insects to behavioral chemicals produced by sympatric bark beetle species. In this study, associated insects responding to various bark beetle pheromone mixtures were monitored by means of replicated field bioassays in the presence and absence of measurable populations of the southern pine beetle.

Materials and Methods

Test Sites, Experimental Design, and Statistical Analysis

Test A. Two traps (described later) for each of five treatments were assigned randomly to 10 trap positions situated in a 25-year-old loblolly pine (*Pinus taeda* L.) forest near Lufkin, in Angelina County, Tex. (Site I). The treatments consisted of pheromone mixtures termed (1) SPB attractant (ASPB), (2) *Ips* attractant (AIPS), (3) SPB inhibitor (ISPB), (4) combinations of *Ips* attractant and SPB inhibitor (AIPS+ISPB), and (5) host-produced volatiles (HV) in the form of loblolly pine turpentine. The chemical compounds, elution devices, and elution rates for test A are listed in Table 1. Insects responding to pheromone treatments were collected on Monday, Wednesday, and Friday from 3 to 24 September 1980. To reduce positional effects, traps and their respective baits were changed systematically to a different position in the trap line following each collection of insects.

From 29 September to 20 October 1980, treatments 1 through 4 were tested again in a Texas Forest Service pine seed orchard near Kirbyville, in Jasper County, Tex. (Site II), to obtain additional replicates using the same formulations, elution devices, and experimental design. For this series of tests, the two traps with host volatiles (treatment 5) were replaced with two traps containing SPB attractant + SPB inhibitor (ASPB+ISPB), using one packet of each behavioral chemical mixture per trap.

To further reduce positional effects and to provide numbers sufficient for statistical analysis, trap-catch data for *M. titillator, T. dubius,* and *T. virescens* were pooled to provide three replicates per treatment at each test site. In this test, a replicate represented the total numbers of a given insect species captured in two traps having the same treatment over a 7-day trapping interval (e.g., Monday to Monday). Data for the bark beetles *I. avulsus, I. grandicollis,* and *I. calligraphus* were recorded every third collection period (e.g., Friday to Monday) at each test site. No *D. frontalis* were trapped at either test site.

In a preliminary analysis of results, catches of *M. titillator, T. dubius,* and *T. virescens* for treatments 1 through 4 at Site I were compared by χ^2 analysis to those for the same treatments deployed at Site II. For each of these associated insects, the distribution of catches among common treatments for Site I was not significantly different ($P > 0.05$) from that obtained in Site II. Thus, data for Site I and Site II were combined by species and treated as six replicates of the same experiment for purposes of statistical analysis (three replicates per site).

To adjust for 0 counts, the raw data for a given species were transformed using the formula $\sqrt{x + 0.5}$, a procedure recommended for pheromone trapping studies (Roelofs and Cardé 1977). The transformed data for each test were then statistically analyzed in a complete randomized block analysis of variance provided by the Statistical Analysis System (Statistical Analysis System Institute 1982). Differences in treatment means were tested for significance at the 0.05 probability level using Duncan's multiple range test.

Test B. A second bioassay was run from 17 August to 7 September 1983, in a recently thinned loblolly pine stand (20 years old) located near Woodville in Tyler County, Tex. (Site III). This test differed from similar bioassays conducted in 1980 (test A; Site I and II) in that populations of *D. frontalis* (in addition to *Ips* spp. and associated insects) were present, having emerged from scattered infested trees in Site III.

Twenty-five traps were used to test five treatments in test B. Treatments consisted of (1) SPB attractant, (2) SPB attractant + SPB inhibitor, (3) *Ips* attractant, (4) *Ips* attractant + SPB inhibitor, and (5) check. Certain formulations and elution rates deployed in test B differed from those used in test A (Table 1). Of specific interest was the use of paste in place of liquid formulations of SPB attractant and liquid instead of paste formulations of SPB inhibitor in test B. Insects were collected at weekly intervals for a 3-week testing period and traps were repositioned randomly at the beginning of each week to account for possible positional effects.

Total numbers of *M. titillator, T. dubius, T. virescens,* and *D. frontalis* responding to a given treatment over the 3-week test period were pooled by species for each of five traps and treated as replicates (e.g., five replicates per treatment). These data were transformed and analyzed by individual species using the same transformation formula and ANOVA procedure described before.

Bioassay Traps

Nonsticky flight traps with funnel collection devices, described in detail by Rose et al. (1981), were baited with candidate compounds. The barrier on each trap was 35 cm wide by 50 cm tall, providing 1,750 cm² of trapping area. Traps were hung at a height of 2 m on nylon cord strung between understory hardwood trees in forest test sites (Site I, III) or between widely spaced pine trees in the seed orchard test site (Site II). Traps were spaced from 20 to 30 m apart under similar stand conditions within each test site.

Table 1. Candidate compounds, formulations, elution containers, and elution rates used in evaluation of southern pine bark beetle behavioral chemicals in test A (1980) and test B (1983) in east Texas

Test	Behavioral description	Compounds	Formulation	Allotment and elution container	Elution rate[a] (mg/day)
A (Site I, II)	SPB attractant (ASPB)	Frontalin + *trans*-verbenol + pine turpentine	1:1:12 Liquid	2 ml, Polyethylene cap	25–40
	SPB inhibitor (ISPB)	Verbenone + *exo*-brevicomin + *endo*-brevicomin	10:9:1 Paste	10 g, Polyethylene bag	75–100
	Ips attractant (AIPS)	Ipsenol + ipsdienol + *cis*-verbenol	1:1:1 Paste	10 g, Polyethylene bag	75–100
	Host volatiles (HV)	Loblolly turpentine	Liquid	2 ml, Polyethylene cap	25–40
B (Site III)	SPB attractant (ASPB)	Frontalin + alpha-pinene	1:2 Paste	10 g, Polyethylene bag	30–60
	SPB inhibitor (ISPB)	*Endo*-brevicomin + verbenone	1:2 Liquid	2 ml, Glass vial with 2-ml hole in cap	10–15
	Ips attractant (AIPS)	Ipsenol + ipsdienol + *cis*-verbenol	1:1:1 Paste	10 g, Polyethylene bag	75–100
	Check	None			

[a] Determined galvametrically under ambient conditions (maximum daily temperatures, 28–34°C).

Behavioral Chemicals

Mixtures of behavioral chemicals were used in field tests since two or more pheromones in combination have proven more promising than single compounds for manipulating southern bark beetle populations (Vité and Francke 1976, Richerson and Payne 1979, Rose et al. 1981). Southern pine beetle attractant in test A consisted of a 1:1:12 liquid mixture of frontalin, *trans*-verbenol, and loblolly pine turpentine, a formulation known to be attractive to flying SPB populations (Payne et al. 1978). *Ips* attractant consisted of 2% ipsenol, 2% ipsdienol, and 2% *cis*-verbenol in 25% pentane (carrier) delivered from a sustained-release formulation consisting of 69% inert vaseline-based paste (J. P. Vité, personal communication), a mixture attractive to the three southern *Ips* spp. (Rose et al. 1981). SPB inhibitor consisted of 2% *exo*, *endo*-brevicomin (ratio = 9:1), 2% verbenone, 26% pentane, and 70% inert vaseline-based paste (J. P. Vité, personal communication).

In test B, the SPB attractant was in the form of a sustained-release paste formulation containing ca. 6% (800 mg) frontalin and alpha-pinene (1:2), provided by Celamerck GMBH and Co. of West Germany. The paste formulation was delivered from individual polyethylene packets. The formulation of *Ips* attractant used in test B was identical to that used in test A. The SPB inhibitor tested in 1983 consisted of a liquid formulation of *endo*-brevicomin and verbenone (1:2). Inhibitor mixtures used in tests A and B were previously found to reduce significantly landing and attack by SPB on treated trees (Richerson and Payne 1979). Bark beetle pheromone compounds were obtained from Chemical Samples Co., unless otherwise indicated. Host-produced volatiles in test A consisted of 100% loblolly pine turpentine.

Elution Devices

Candidate compounds were eluted by evaporation from various elution devices (Table 1). In test A (Site I and II), the SPB attractant and host volatiles were deployed by placing 2 ml of the liquid formulations in small polyethylene caps. The caps were for Kimble 60975-L 7-ml vials, no. 3-33910B, Fisher Scientific Co. One filled cap was placed within the polyethylene sleeve of each test trap. The paste formulations of *Ips* attractant and of SPB inhibitor were deployed by placing 10 to 15 g in a 7-by-10-cm polyethylene zip lock bag, one bag per trap. For tests requiring a combination of attractant and inhibitor, one bag of each was used per trap. The paste formulations of SPB attractant used in test B were in 6-by-8-cm polyethylene packets and one packet per trap was used. In test B, the liquid formulation of SPB inhibitor was deployed from 7-ml capped glass vials with a 2-mm opening drilled in the cap.

Results

D. frontalis. No *D. frontalis* were trapped at any of the treatments in test A (Site I or Site II) conducted during 1980, reflecting the extremely low population levels of this bark beetle in Texas during this period (Texas Forest Service 1982).

With the return of high population levels in 1983, a total of 802 *D. frontalis* was trapped in test B. Of these, 790 (99%) were caught in traps baited with SPB attractant or SPB attractant plus SPB inhibitor (Fig. 1). The addition of SPB inhibitor to traps also containing SPB attractant did not reduce catches, presumably due to the slow release rate of SPB inhibitors used in test B. On the contrary, in four of five replicates *D. frontalis* exhibited greater responses to traps containing SPB attrac-

tant and SPB inhibitor than to traps with SPB attractant alone. Differences between these two treatments, however, did not prove significant ($P > 0.05$). More males than females responded to the traps baited with frontalin (Fig. 1), confirming earlier findings (Renwick and Vité 1970, Payne et al. 1978).

***Ips* spp.** In test A, *I. calligraphus*, *I. grandicollis*, and *I. avulsus* responded in sizeable numbers only to those treatments containing *Ips* attractant (Fig. 1). Similar results were observed in test B, but actual numbers were not recorded. In test A, the addition of SPB inhibitor to traps baited with *Ips* attractant significantly reduced catches for *I. avulsus* ($P < 0.05$), but had no effect on *I. grandicollis* or *I. calligraphus*. Only occasional *Ips* beetles were caught in traps baited with SPB attractant and/or SPB inhibitor or with host volatiles alone.

***M. titillator*.** In test A (Table 2) and test B (Table 3), *Monochamus titillator* responded in significant numbers to traps containing *Ips* attractant, but not to traps containing SPB attractant. Males and females were drawn to *Ips* attractant in similar numbers. Only ca. 2% of the total *M. titillator* trapped in either test A or test B were caught in traps baited solely with SPB attractant, numbers that were not significantly different from 0 ($P > 0.05$). No sawyers were caught in traps baited with host-produced volatiles or SPB inhibitors *per se* in test A. Interestingly, the addition of SPB inhibitor to traps containing *Ips* attractant in test A appeared to increase the response of both male and female *M. titillator*, a finding that was confirmed in test B. This apparent synergistic effect was observed with both high (test A) and low (test B) release rates of SPB inhibitors (see Table 1), but proved statistically significant ($P < 0.05$) only in test B. Also in test B, a combination of SPB attractant and SPB inhibitor attracted *M. titillator*, but in significantly fewer numbers ($P < 0.05$) than did Ips attractant alone.

***T. dubius*.** In test A, conducted during 1980, *T. dubius* responded to SPB attractant as well as to *Ips* attractant at both test sites, but showed a significant preference for SPB attractant (Table 2). Females and males responded in a 1:1 ratio to SPB attractant and in a 1.3:1 ratio to *Ips* attractant. The addition of SPB inhibitor to traps baited with SPB attractant in test A significantly reduced catches of *T. dubius* ($P < 0.05$). A similar decrease in response occurred in test A when *Ips* attractant was offered in combination with SPB inhibitors compared to *Ips* attractant alone.

The preference of *T. dubius* for traps baited with SPB attractant as compared to *Ips* attractant was even more evident in test B (Table 3). Of 174 *T. dubius* caught in all treatments, 89% responded to traps baited with either SPB attractant or SPB attractant in combination with SPB inhibitor. In test B, the addition of SPB inhibitor to traps baited with SPB attractant or *Ips* attractant had no significant effect on the response of *T. dubius*, presumably due to the lower release rate of the inhibitor compared to that of test A.

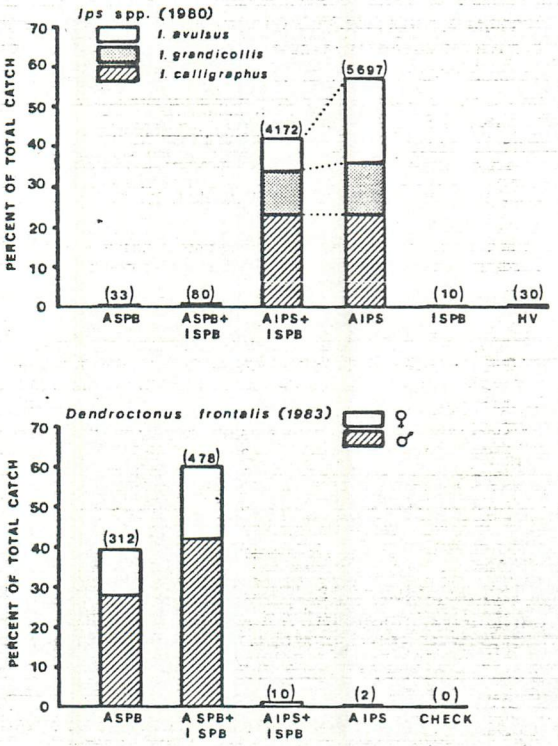

Fig. 1. Relative responses of southern *Ips* spp. (1980) and *D. frontalis* (1983) to traps baited with mixtures of synthetic behavioral chemicals in east Texas. ASPB, southern pine beetle attractant; ISPB, southern pine beetle inhibitor; AIPS, *Ips* attractant; HV, host volatiles (pine turpentine); and check, bare trap as defined in Table 1.

In 1980, when populations of *D. frontalis* apparently were not present in the area, 26% of all *T. dubius* captured in test A responded to traps baited with *Ips* attractant only (Table 2). However, in 1983, following a buildup of *D. frontalis* populations in the test area, only 2% of the total *T. dubius* catch in test B responded to *Ips* attractant (Table 3). Indeed, the numbers of *T. dubius* responding to *Ips* attractant in test B were not significantly different from the check ($P > 0.05$).

***T. virescens*.** In contrast to *Thanasimus dubius*, the predator *Temnochila virescens* responded in numbers significantly greater than the check ($P < 0.05$) only to treatments containing *Ips* attractant (Table 2). In test A, only 3% of the total catch of *T. virescens* responded to traps baited with SPB attractant at either test site. Similar results were found in test B (Table 3). The addition of SPB inhibitor to traps with *Ips* attractant did not significantly reduce total catches of *T. virescens* in either test A or test B. Sex ratios of responding beetles were 0.7 females per male to *Ips* attractant and 0.9 females per male to *Ips* attractant + SPB inhibitor.

Table 2. Responses of *M. titillator*, *T. dubius*, and *T. virescens* in test A to mixtures of southern pine bark beetle behavioral chemicals deployed in a loblolly pine forest (Site I) and a pine seed orchard (Site II) in east Texas, 1980

Treatment[a]	No. replicates	*M. titillator* Total catch[b]	*M. titillator* Mean catch per rep. ± SE	*T. dubius* Total catch	*T. dubius* Mean catch per rep. ± SE	*T. virescens* Total catch	*T. virescens* Mean catch per rep. ± SE
ASPB	6	2	0.3 ± 0.2b	345	57.5 ± 17.8a	3	0.5 ± 0.2b
ISPB	6	0	0b	0	0c	0	0b
AIPS	6	30	5.0 ± 1.8a	145	24.2 ± 6.9b	60	10.0 ± 3.5a
AIPS + ISPB	6	54	9.0 ± 3.0a	4	0.7 ± 0.3b	34	5.7 ± 2.1a
ASPB + ISPB[c]	3	3	1.0 ± 1.0b	59	19.7 ± 9.2b	0	0b
HV[d]	3	0	0b	1	0.3 ± 0.3c	1	0.3 ± 0.3b
		Σ = 89		Σ = 554		Σ = 98	

Means followed by the same letter are not significantly different ($P < 0.05$; Duncan's multiple range test).
[a] ASPB, Southern pine beetle attractant; ISPB, southern pine beetle inhibitor; AIPS, *Ips* attractant; HV, host volatiles as defined in Table 1 (test A).
[b] Total catch for three 1-week trapping intervals (replicates) at each test site.
[c] Tested at Site II only.
[d] Tested at Site I only.

Discussion

The response of *M. titillator* to *Ips* attractant is particularly noteworthy since this is the first documentation of sawyer beetles exhibiting a kairomonal response to bark beetle pheromones. The ability of *M. titillator* to respond to either *Ips* attractant or a combination of SPB attractant and SPB inhibitor assures that these wood-boring insects will locate suitable breeding material regardless of which bark beetle predisposes the tree.

The predator *T. dubius* is known to respond to frontalin in the field (Vité and Williamson 1970, Dixon and Payne 1980) and to ipsdienol and to *trans*-verbenol in the laboratory (Mizell 1980). Results from the present study support earlier findings (Dixon and Payne 1979, Payne et al. 1984) that the response behavior of *T. dubius* is preferentially synchronized with the bark beetle *D. frontalis*, considered to be its preferred host.

Populations of *D. frontalis* are typically cyclic and may be largely unavailable as hosts for this predator during certain years. The ability to respond to pheromones produced by the more ubiquitous *Ips* beetles accommodates survival of *T. dubius* when its preferred host is unavailable. As suggested for certain southern pine beetle parasites (Kudon and Berisford 1980), the perennial availability of *Ips* beetles may be the key to maintaining reservoirs of natural insect enemies during periods of low *D. frontalis* populations.

The *T. dubius* adults trapped in the 1980 tests presumably were reared from *Ips*-killed trees since no confirmed *D. frontalis* infestations were detected in the counties where these tests were conducted (Texas Forest Service 1982). Accordingly, the increased response of *T. dubius* to *Ips* attractant in 1980 tests (26%) compared to that observed in 1983 tests (2%) suggests that the response behavior of this predator may vary depending on the bark beetle host upon which it was reared. This hypothesis warrants further study.

Monochamus titillator, a competitor, and *T. virescens*, a predator, are both commonly observed on trees killed by *D. frontalis* (Dixon and Payne 1979, Hennier 1983). In contrast to *T. dubius*, however, these species apparently have not adapted to the aggregating pheromone of *D. frontalis*, but respond to *Ips* pheromones as a means to locate prey or suitable host material for oviposition. *Temnochila chloridia* Mannerheim, a species common to western pine forests, is known to respond to *exo*-brevicomin, a pheromone produced by males of the western pine beetle, *D. brevicomis* LeConte (Wood et al. 1968). In the present study, *T. virescens* showed no response to treatments containing only brevicomin and verbenone.

The kairomonal response preferences of *T. dubius*, *T. virescens*, and *M. titillator* reported here help explain the observed arrival sequence of these insects to bark-beetle-infested trees. Camors and Payne (1973) and Dixon and Payne (1979) have observed that *T. dubius* arrives at host trees almost simultaneously with *D. frontalis*. Clearly, both predator and prey respond to the attractant frontalin in combination with host odors released at the time of initial *D. frontalis* attack. In the present study, the presence of high levels of SPB inhibitor (test A) reduced the response of *T. dubius* to traps baited with either SPB or *Ips* attractant. An inhibitory reaction to SPB inhibitor coincides with the observation that *T. dubius* adults are common only on trees undergoing active *D. frontalis* attack (Camors and Payne 1973, Dixon and Payne 1979). Yet, Richerson and Payne (1979) found that the application of synthetic SPB inhibitor to trees under bark beetle attack significantly reduced landing rates of *D. frontalis*, but not those of *T. dubius*.

Temnochila virescens was observed by Dixon and Payne (1979) to arrive at two periods during the bark beetle colonization period of southern pines. These coincided with peak arrivals of *I. avulsus* (day 9–12) and *I. calligraphus* (day 22–30). Few *T. virescens* arrived before day 7, when

Table 3. Responses of *M. titillator*, *T. dubius*, and *T. virescens* in Test B to mixtures of southern pine bark beetle behavioral chemicals deployed in a thinned loblolly pine stand (Site III) in east Texas, 1983

Treatment[a]	No. replicates[b]	*M. titillator*		*T. dubius*		*T. virescens*	
		Total catch	Mean catch per rep. ± SE	Total catch[c]	Mean catch per rep. ± SE	Total catch	Mean catch per rep. ± SE
ASPB	5	4	0.8 ± 0.4d	77	15.4 ± 5.4a	6	1.2 ± 0.5b
AIPS	5	42	8.4 ± 2.0b	4	0.8 ± 0.4b	60	12.0 ± 1.9a
AIPS + ISPB	5	142	28.4 ± 2.3a	15	3.0 ± 2.3b	45	9.0 ± 2.3a
ASPB + ISPB	5	24	4.8 ± 1.1c	78	15.6 ± 5.9a	8	1.6 ± 0.7b
Check	5	0	0d	0	0b	1	0.2 ± 0.2b
		Σ = 212		Σ = 174		Σ = 120	

Means followed by the same letter are not significantly different ($P < 0.05$; Duncan's multiple range test).
[a] ASPB, southern pine beetle attractant; ISPB, southern pine beetle inhibitor; AIPS, *Ips* attractant as defined in Table 1 (test B).
[b] Each replicate equals catch in one trap of a given treatment over a 3-week trapping interval.
[c] Total catch for five traps tested over a 3-week trapping interval.

D. frontalis pheromones predominate. The response of *T. virescens* to pheromones produced by attacking *Ips* species and lack of response to *D. frontalis* attractant explain this temporal arrival pattern.

Adults of *M. titillator* arrive primarily from 1 to 10 days following successful attack by *D. frontalis* (Hennier 1983). Present findings confirm that the early arrival of *M. titillator* to bark-beetle-infested trees occurs in response to behavioral chemicals produced by coinhabiting bark beetle species. Peak response is likely to occur from 4 to 10 days after initial *D. frontalis* attack—the interval when a combination of SPB inhibitor and *Ips* attractant predominates.

By responding to bark-beetle-produced behavioral chemicals, male and female *M. titillator* adults are brought into proximity for mating and females are assured suitable sites for oviposition. Whether this species produces its own behavioral chemicals remains unknown.

Finally, since mixtures of several pheromone components attractive to *Ips* were used in this study, it remains to be determined whether responses by *M. titillator*, *T. dubius*, and *T. virescens* were elicited by a single pheromone component or by a synergistic combination of components. Bakke and Kvamme (1981) report that *Thanasimus formicarius* (L.) and *Thanasimus femoralis* (Zetterst) respond to racemic ipsdienol and ipsenol and less to (S)-*cis*-verbenol, pheromone components produced by several *Ips* bark beetles that serve as prey. Synergistic effects appeared when the single components were combined. Particularly in the case of *T. dubius*, kairomonal response patterns may parallel those documented for these two European counterparts.

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